Remarks and Arguments

Claims 1-22 and 30 and 31 directed to methods to determine viral loads have been canceled without prejudice. Claims 23-29 and 32 remain pending and new claims 33-62 have been added, all directed to methods and kits to determine mitochondrial toxicity. Applicants reserve the right to continue prosecution of any previously claimed subject matter in a continuation application. Applicants have amended claim 23 without prejudice to promote prosecution. New claims 33-62 directed to (i) characterization of the host nuclear nucleic acid find support for example on page 18, lines 11-18, (ii) specific sequences useful in primer/probe design find support on pages 33 and 34, and (iii) kits for the assessment of toxicity on pages 49-50 of the specification.

Rejection of Priority Claim

Applicants understand that the Examiner has apparently rejected the priority claim of the pending application because the listed provisional applications allegedly do not disclose a method in which nucleic acids are contacted with an amplification mixture containing primers/probes that "provide a detectable signal" for both viral and host nucleic acid transcription to accomplish "identifying a compound which inhibits viral replication" as required by claims 1-22. While Applicants disagree with the Examiner, claims 1-22 have been cancelled and thus the rejection of the priority of these claims is moot.

It also appears that the Examiner has rejected the priority claim of claims 23-32 because the priority applications supposedly did not disclose a method in which nucleic acids are contacted with an amplification mixture containing primers/probes that "provide a detectable signal" for both mitochondrial and nuclear nucleic acid amplification to accomplish "assessing the toxicity of a compound" as required by claims 23-32. Applicants respectfully disagree with the Examiner's findings.

Specifically, Applicants point to Provisional Application No. 60/256,067 (the '067 application), which discloses each of the points in question. The '067 application specifically discloses a method for determining the mitochondrial toxicity of a compound by determining the ratio of mitochondrial to nuclear nucleic acids (see page 4, line 20 to page 6, line 18, and page 10, lines 6 to 13). This method includes: contacting a sample with an amplification reaction

mixture (page 6, lines 3 to 4) that contains at least one probe that provides a detectable signal when contacting nuclear nucleic acid sequences (page 6, lines 6-8) and at least one probe that emits a detectable signal when contacting mitochondrial nucleic acid sequences (page 6, lines 15-17) to "determine toxicity of potential anti-retroviral agents" (page 4, line 23 to page 5, line 1). Applicants submit that the steps required for claims 23-32 are thus identified in this provisional application and should be afforded a filing date of December 15, 2000.

Rejections under 35 USC § 112

The Examiner has rejected originally pending claims 1, 6, 10-11, 23, 26, and 30-31 under 35 USC § 112, first paragraph, because the specification allegedly does not provide enablement for methods in which the viral or mitochondrial genes are from β-actin or GAPDH. Claims 1, 6, 10, 11, and 30-31 have been canceled. Independent claim 23 and dependent claim 26 do not specifically refer to β-actin or GAPDH. The rejection of these claims is thus moot. New claims 33-45 have been added to further specify the host nuclear nucleic acid sequences, and claims 39 and 40 have been added to specify that the target nuclear host genes are β-actin or GAPDH, respectively.

The Examiner has rejected original claims 1-32 under 35 USC § 112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Claims 1-22 are canceled and the rejections of these claims are thus moot. Claims 23-32 were rejected as indefinite because it was purportedly unclear as to how the practice of the method steps required by the claims allows one to achieve the objective of "assessing the toxicity of a compound," as recited in the preamble of claim 23. The amended claims are limited to detecting mitochondrial toxicity by comparing the first and second detectable signals. Amended claim 23 finds support throughout the specification, for example on page 19, lines 3-5 and 62-63, bridging paragraph. Applicants believes the rejection is therefore overcome.

The Examiner has rejected original claims 23-32 as indefinite, apparently due to the recitation of the phrases "at least two primers and/or probes," "the first primer and/or probe," and "the second primer and/or probe" in claim 23. The claims have been simplified to refer to "a first" and "a second" primer with detectable signals. Applicants have amended claim 23 to recite a primer rather than "primer and/or probe" for clarity. On page 31 of the originally filed application, the term "probe" is defined on lines 14-24. The probe is defined as "an

oligonucleotide having a specific or desired nucleotide sequence that is complementary to a particular sequence on one of the strands of a DNA duplex." To this point, the definition of the term "probe" appears identical to that for the term "primer" (see page 31, lines 1-13), however a "probe" also includes "a detectable signal" (page 31, line 16). Therefore, Applicants believe the phrase "primers that provide a detectable signal" as required for amended claim 23 includes a probe and use of the term in the claim would therefore be redundant.

The Examiner has further rejected original claims 23-32 as indefinite because the term "occurrence of transcription" lacks antecedent basis. The claims have been amended to read "upon amplification" of nucleic acids. This finds support throughout the specification, for example in the description of the term "probe" on page 31. The Examiner has also alleged that original claims 24-32 are indefinite apparently due to the recitation of "the host mitochondrial nucleic acid" in claims 24-26 and 32. The Examiner suggested that there is insufficient antecedent basis for this recitation in the claims, as claim 23 refers to "host mitochondrial nucleic acids," but not to a "host mitochondrial nucleic acid." To overcome the rejection, the claim has been amended as suggested by the Examiner to refer to a host mitochondrial nucleic acid.

Rejections under 35 USC §102

The Examiner has rejected original claims 1-2 and 12 under 35 USC § § 102(a) and 102(e) as anticipated by Hall *et al.* (U.S. Patent No. 6,218,105 B1). The Examiner has also rejected original claims 6-9 under 35 USC §§ 102(a) and 102(e) as anticipated by Hall *et al.* in light of the teachings of Crooke *et al.* (U.S. Patent No. 5,756,282), and claim 11 under 35 USC § § 102(a) and 102(e) as being anticipated by Hall *et al.* in light of the teachings of Ercolani *et al.* (J. Biol. Chem. 263(30):15335-15341 (1988)).

Applicants have canceled claims 1-22, which were directed to assessment of viral load. Applicants have also amended pending claims 23-32 and added new claims 33-56, which are directed to the assessment of mitochondrial toxicity rather than assessment of viral load. The rejections of original claims 1-22 under 35 USC §§ 102(a) and 102(e) are therefore moot.

Rejections under 35 USC § 103

The Examiner has rejected claims 3-5 under 35 USC § 103(a) as obvious in light of the combination of Hall *et al.* (U.S. Patent No. 6,218,105) in view of Pan Zhou *et al.* (Antimicrob.

Agents Chemother. 44(3):496-503 (2000)). The Examiner has also rejected claim 10 under 35 USC § 103(a) over Hall *et al.* in light of the teachings of Ng *et al.* (Mol. Cell. Bio. 5(10):2720-2732 (1985)), and claims 13-22 under 35 USC § 103(a) as being unpatentable over Hall *et al.* in view of Barney *et al.*(U.S. Patent No. 6,054,265 (2000)).

Claims 1-22 are canceled and these rejections are therefore moot.

Thomas et al. in combination with Hall et al.

The Examiner has rejected claims 23-25 and 32 under 35 USC § 103 (a) as obvious in light of Thomas *et al.* (Clin. Sci. 97:207-213 (1999)) in view of Hall *et al.* (U.S. Patent No. 6,218,105). Thomas *et al.* investigate the effects of ethidium bromide (EtBr) treatment of immortalized thyroid cells on nuclear gene expression. It was known at the time that ethidium bromide completely depletes mitochondrial DNA. These investigators studied how the "complete and permanent loss" of mitochondrial DNA (page 208) affects nuclear gene transcription. Since the investigators were working with cells that have lost mitochondrial DNA, they were not, by definition, assessing the toxicity of a drug by comparing nuclear and mitochondrial DNA, because the cell no longer had mitochondrial DNA. The article does not suggest that the comparison of nuclear to mitochondrial nucleic acids be used to assess the incremental toxicity of a drug on the mitochondria, because according to the Thomas test design, the mitochondrial DNA was destroyed. Only the nuclear DNA was assessed by Thomas *et al.*

Hall *et al.* measures the expression of at least one viral nucleic acid in cells as a means to to assess the activity of test compounds against papilloma virus using RT-PCR. Host cell nucleic acid is detected as an internal mRNA control (column 3, line 38-39) to quantitate viral load. Hall actually supports the nonobviousness of the present claims. Hall claims a priority date of January 8, 1999. It has been known since at least 1991 that that antiviral drugs, and in particular, AZT, can cause peripheral neuropathy due to mitochondrial toxicity (Chen *et al.* Mol. Pharmacol. 39:625-8 (1991)). The technique employed by Chen *et al.* in investigating the effects of various anti-viral compounds on mitochondrial DNA was extremely cumbersome and required several days to provide a result. The steps required included isolating DNA from cells, digesting the DNA with a restriction enzyme, separating the fragments on an agarose gel, transferring the DNA to a nitrocellulose membrane, and probing the membrane for mitochondrial cytochrome oxidase. At no time did the Chen technique allow for amplification of the DNA.

Furthermore, the mixture used for probing contained only a single DNA to the mitochondrial gene, although in a separate step, the nitrocellulose was probed for topoisomerase to adjust for loading of the gel. However, the technique used by Chen in 1991 was plainly complex, time consuming, hazardous, and likely error prone.

Given that Hall used host cell nucleic acid as an internal mRNA control to measure viral load against, if it were so obvious to extend the Hall technique to measuring mitochondrial DNA to assess mitochondrial toxicity, presumably Hall in 1999, Cheng in 1999, or someone else would have done or described that. It was in fact not done or described until the filing of the present application. In fact, as late as 1998, Cheng's laboratory was using a similar technique as in 1991 (see Dutschman *et al.* Antimicrob. Agents Chemother. 42:1799-1804 (1998)). Scientists simply did not consider a method of using two detectable primers to compare mitochondrial and nuclear nucleic acid amplification to assess the toxicity of a compound. The fact that almost ten years spanned between the discussion of mitochondrial toxicity of antiviral nucleosides in the literature and the disclosure of the present method despite burgeoning research in this field is very strong evidence of nonobviousness.

To support a rejections under 35 USC § 103, three criteria must be met: 1) there must be a motivation to combine the references, either in the art or in the references; 2) there must be a reasonable expectation of success in the combination; and 3) all the limitations of the claimed inventions must be found in the references. *In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The three criteria that are required to uphold a rejection under 35 USC §103 are not met in the combination of Hall *et al.* and Thomas *et al.* First, there is no suggestion that these methods be combined. Second, there is no indication that combining the methods of Hall *et al.* and Thomas *et al.* would result in successful assessment of drug toxicity. Specifically, Hall *et al.* discloses the use of primers that are directed to the splice site, or intron-exon boundary, of viral DNA and genomic DNA as an internal control to be compared to the viral genes (see Figure 2; Figure 3a; clm 7, line 64). Thomas *et al.* describes the nuclear effects that are associated with drug-induced complete loss of mitochondrial DNA. There is no indication in these references that the comparison of mitochondrial to nuclear nucleic acid using any technique, or in particular a technique similar to that used in Hall, would succeed in assessing the toxicity of a compound, as recited in the preamble of claim 23. The Examiner appears to argue that Thomas *et al.* provides a suggestion that there are genes that may only be marginally differentially expressed in

treated cells but that may still be of pathological significance (page 212, second paragraph). However, we disagree with the Examiner's interpretation of these statements. In the article, the authors are referring to the difference between nuclear gene transcription in treated and untreated cells, they are not referring to comparing mitochondrial to nuclear gene transcription or amplification. Furthermore, the Examiner points to the suggestion that further studies be conducted to confirm whether the *nuclear* genes that were identified as differentially expressed by Thomas *et al.* "are similarly affected" in other cell lines. Again, mitochondrial toxicity of EtBr was not assessed because it was known that it results in the "complete and permanent loss" of mitochondrial nucleic acids. Lastly, the references do not teach "all the limitations of the claimed inventions" as required to support a rejection under 35 USC § 103 as neither reference discloses a method for "assessing the mitochondrial toxicity of a compound" as defined in the preamble of claim 23. The combination of these references does not anticipate or render obvious the claimed invention.

Thomas et al. in combination with Hall et al. in combination with Ojala et al.

The Examiner has also rejected claims 26-29 under 35 USC § 103(a) as obvious in light of Thomas *et al.* in view of Hall *et al.*, in light of teachings of Ojala *et al.*(Nature 290:470-474 (1981)). As described above, the combination of Thomas *et al.* and Hall *et al.* does not support a rejection under 35 USC § 103(a). Ojala describes a model in which the full length "H"-strand of mitochondrial DNA is transcribed into contiguous *coding* sequences. The Ojala model simply describes how mitochondrial nucleic acid is transcribed. Applicants fail to undertand how that renders the claimed invention obvious, given that the independent claim is nonobvious. The CAFC has held that if an independent claim is non-obvious under 35 USC § 103, dependent claims thereof are also non-obvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988). Ojala is not relevant.

Thomas et al. in combination of Hall et al. in combination with Ng et al.

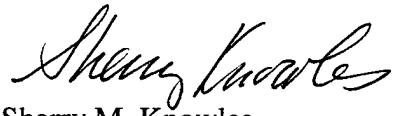
The Examiner has also rejected claims 30 and 31 under 35 USC § 103(a) as nonobvious over Thomas *et al.* in view of Hall *et al.*, in light of the teachings of Ng *et al.*(Mol. Cell. Biol. 5(10):2720-2732 (1985)), or in light of the teachings of Ercolani *et al.*(J. Biol. Chem. 263(3):15335-15341 (1988)), respectively. Ng described the ß-actin gene and Ercolani describes

GAPDH. Again, the CAFC has held that if an independent claim is non-obvious under 35 USC § 103, dependent claims thereof are also non-obvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

Conclusion

Based on the above-presented amendments and arguments, Applicants request that the Examiner allow all pending claims. Should the Examiner have any questions about the pending claims he is invited to contact the undersigned at 404-572-3541. The Commissioner is authorized to charge any additional fee or credit any overpayment associated with this submission, to Deposit Account No. 11-0980.

Respectfully submitted,



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